A novel and more efficient biosynthesis approach for human insulin production in *Escherichia coli (E. coli)*.

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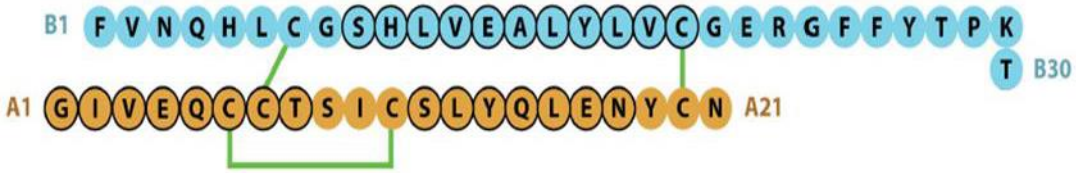

Figure 1: Insulin structure depicting polypeptide chains A and B, the A chain is depicted in brown, the B chain is depicted in blue, and the disulphide bonds are depicted in green, adapted from [1] (open access).

Insulin has captured researchers' attention worldwide. There is a rapid global rise in the number of diabetic patients, which increases the demand for insulin. Current methods of insulin production are expensive and time-consuming. A PCR-based strategy was employed for the cloning and verification of human insulin. The human insulin protein was then overexpressed in *E. coli* on a laboratory scale. Thereafter, the optimisation of human insulin expression was conducted. The yield of human insulin produced was approximately 520.92 (mg/L), located in the intracellular fraction. Human insulin was detected using MALDI-TOF-MS and LC-MS methods. The crude biosynthesised protein sequence was verified using protein sequencing, which had a 100% similarity to the human insulin sequence. The biological activity of human insulin was tested *in vitro* using a MTT assay, which revealed that the crude biosynthesised human insulin displayed a similar degree of efficacy to the standard human insulin. This study eliminated the use of affinity tags since an untagged pET21b expression vector was employed. Tedious protein renaturation, inclusion body recovery steps, and the expensive enzymatic cleavage of the C-peptide of insulin were eliminated, thereby making this method of biosynthesising human insulin a novel and more efficient method.

Keywords:

Biosynthesis of human insulin; diabetes; *E. coli*.

References:

1. Lawrence, M., *Landmarks in insulin research.* Frontiers in Endocrinology, 2011. **2**: p. 76.